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PROPERTIES OF A MONOCLONAL ANTIBODY INTERACTING WITH HUMAN SPERM

YUAN CHANG YAN, LIN FANG WANG, and S. S. KOIDE

A monoclonal antibody (MAB) interacting with human sperm was purified by ammonium sulfate fractionation and gel filtration on Sephadex G-200. The MAB designated as YWK-II belongs to the IgM class and is composed of μ heavy chains and κ light chains. The MAB localized to the equatorial region of the sperm head, midpiece, and tail. The sperm antigen interacting with the YWK-II MAB was purified by immunoaffinity chromatography on Sepharose 4B conjugated with YWK-II antibodies. Two interacting proteins, estimated Mr of 60 and 72 kD, were identified.

Key Words: Human sperm; Monoclonal antibodies; Immunologic infertility; Sperm agglutination.

INTRODUCTION

Antisperm antibodies have been implicated in immunologic infertility [1, 4, 8–10, 12, 15, 18, 22, 24, 30, 31, 34, 37]. The antibodies involved are those that induce sperm agglutination and/or immobilization [19, 21]. Although these antibodies are found in the sera, cervical fluid, and seminal plasma obtained from individuals with immunologic infertility, the titers are variable and a cause and effect relationship has not been established. These antibodies probably act by impeding sperm migration through the cervical mucus and vaginal or uterine passages and interfering with sperm-egg interaction (fertilization) [3, 6, 7, 13, 23, 31, 32].

To resolve the issue that the production of a specific antisperm antibody can cause immunologic infertility, the sperm antigen needs to be identified, purified, characterized, and shown to cause infertility after inoculation into an appropriate host animal [29, 38]. The present study was carried out to characterize a monoclonal antibody (MAB) that induces sperm agglutination and to identify the interacting sperm antigen.

MATERIALS AND METHODS

Methods of preparation of human sperm membrane proteins (HSMP), MABs, cloning of the hybridoma cells, and production of ascitic fluid were described in a previous publication [39].

Measurement of Sperm Agglutinating Activity. Sperm agglutinating activity was determined by the

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microtray test as described by Marmar et al. [27]. YWK-II ascitic fluid and SP 2/0 ascitic fluid (control cells) were serially diluted from 1:10 to 1:2430. Sperm concentration was determined by counting in a hemocytometer. Fresh semen was diluted with Baker's buffer pH 7.8, to a final count of 4×10^7 spermatozoa and used immediately. The diluted samples (5μl) were mixed with 1 μl of sperm suspension in a well of a tissue culture plate overlayered with paraffin oil. The plate was incubated at 37 °C for 90 min and examined under an inverted microscope for agglutination of sperm.

Identification of Class and Subclass of YWK-II Immunoglobins. A. Determination of immunoglobulin class by double diffusion assay. Purified immunoglobulins isolated from the medium of cultured YWK-II cells were analyzed in a 1%-agarose Ouchterlony plate with antibodies raised against the various classes of immunoglobulins. Purified YWK-II immunoglobulin solution (20μl) was placed in the center well. The peripheral wells contained 20 μl each of the following antisera: (1) goat antimouse IgM; (2) goat antimouse IgM, diluted 1:10; (3) goat antimouse IgG; (4) goat antimouse IgG, diluted 1:10; (5) goat antimouse IgA; (6) goat antimouse IgA, diluted 1:10.

B. Determination of subclass of Ig by enzyme-linked immunosorbent assay (ELISA). The assay system is described in our previous paper [40]. In summary, wells of a microtiter plate were coated with 5 μg of sperm membrane proteins. The plates were incubated with YWK-II immunoglobulin at ambient temperature for 2 h. After blocking with a solution of 1% BSA and washing, rabbit antibodies raised against the various subclasses of mouse heavy and light chains were added. After 2 h of incubation, peroxidase-conjugated goat antirabbit IgG antibody was added. After incubation for 3 h, ABTS-H_20_2 solution was added. The plate was incubated for 30 min and the absorbance measured at 409 nm in a Bio-Tek Microplate Reader.

Purification of YWK-II MAB. The immunoglobulins obtained from the medium of cultured YWK-II cells were fractionated by a 3.5M ammonium sulfate precipitation and by gel filtration on a Sephadex G-200 column. Fractions of 2 ml were collected at a flow rate of 12 ml/h. Absorbance of each tube was measured at 280 nm. All procedures were performed at 4 °C.

Sodium Dodecyl Sulfate Polyacrylamide Electrophoresis (SDS PAGE). The procedure described by Laemmli [25] was followed. Samples of partially purified immunoglobulins were heated at 100 °C for 5 min in 2% SDS and 5% mercaptoethanol (v/v). Untreated samples were run on the same slab gel. Stacking and separating gels composed of 5% and 12.6% acrylamide, respectively, were used. Proteins were strained with silver reagent.

Immunoblotting Analysis. Method described by Towbin et al. [36] was followed.

Preparation of Sepharose 4B Conjugated with YWK-II MAB. CNBr-activated Sepharose 4B (0.5 g) (Pharmacia) was washed and suspended in 0.1 M $NaHC0_3$, 0.5 M NaCl, pH 8.3, and mixed with 3 ml of YWK-II ascitic fluid. The mixture was rotated end-over-end at 28 °C for 1 h, then stored overnight at 4 °C, and poured into a column (0.9 × 1.8 cm). The Sepharose column was washed with the following media sequentially: 1 M Tris-HCl buffer, pH 9.0; 0.1 M acetate buffer, 1 M NaCl, pH 4.0; 0.1 M borate buffer, 1 M NaCl, pH 8.0; repeated washing with acetate and borate buffer twice; finally, 0.15 M NaCl, 0.01 M PBS buffer, pH 7.4.

Immunoaffinity Chromatography. A sample of HSMP (3 mg) dissolved in 0.15 M NaCl, 0.01 M PBS buffer, pH 7.4, was applied to the column containing 0.5 g of Sepharose 4B conjugated with the YWK-II antibodies and stored overnight at 4 °C. The column was eluted with PBS buffer, followed with 3.5 M KI. Fractions of 1 ml/tube were collected at a flow rate of 6 ml/h.

Sperm Penetration of Zona-Free Hamster Egg Assay. Semen samples from normal fertile donors were left to liquify, standing at 37 °C for 30 min. The motile sperm were centrifuged at 500 × *g* for 5 min and washed three times by suspensions in Biggers, Whitten, and Whittingham's medium (BWW)

[5]. Aliquots of the sperm suspension were incubated at 37 °C in a 5% CO_2 atmosphere for 2 h to permit capacitation to occur. The sperm were counted in a hemocytometer and the suspension adjusted to 1×10^7 sperm/ml.

Sexually mature (8–20 weeks old) golden hamsters were injected intraperitoneally with 40 iu of pregnant mare's serum and then 40 iu of hCG 48 h later. The hamsters were sacrificed 16 h after the last injection by cervical dislocation. The egg masses were dissected out and treated with 0.1% hyaluronidase for 15 min and 0.1% trypsin for 1 min to remove the cumulus and zona. The eggs were placed in a drop of BWW medium in a 35-mm Pertri dish and overlayered with sterile paraffin oil.

Aliquots (20 μl) of the incubated sperm suspensions were mixed with 5 μl of MAB ascitic fluid, control SP 2/0 ascitic fluid or medium. After 10 min the sperm suspensions were transferred to the dishes containing 10 eggs under oil and the dishes incubated at 37 °C in a CO_2 incubator. Ovum penetration was measured at 3 h by examination under an inverted microscope and scored by the formation of pronuclei.

RESULTS

Sperm Agglutinating Activity of YWK-II MAB

The YWK-II ascitic fluid (1:30 dilution) was added to a suspension of human sperm. Sperm agglutination of a mixed type was elicited (Fig. 1).

Immunolocalization of YWK-II Antibody on Human Sperm

Localization of the YWK-II antibodies on human sperm was demonstrated by an indirect immunofluorescence method. Intense staining occurred over the equatorial region of the head, midpiece, and tail (Fig. 2).

Interaction of YWK-II MAB with Sperm Proteins

The interaction of YWK-II ascitic fluid with human sperm proteins was assayed by the ELISA method. Positive reaction was obtained with ascitic fluid diluted 1:900.

Electrophoretic Separation of YWK-II Immunoglobulins

The culture medium containing YWK-II cells was collected and fractionated by precipitation with ammonium sulfate and by gel filtration on a Sephadex G-200 column. The purified YWK-II MAB was analyzed by SDS PAGE under reduced and nonreduced conditions (data not shown). The purified YWK-II MAB migrated under nonreduced conditions as a single band with an estimated Mr exceeding 900 kD. When the purified MAB was analyzed under reduced conditions, three major bands corresponding to the heavy and light chains and $2\mu2\kappa$ chains of IgM were found with estimated Mr of 75, 22, and approximately 220 kD, respectively.

Identification of Class and Subclass of YWK-II Immunoglobulin

Immunoglobulin class typing of YWK-II MAB was performed with the respective specific antibodies in a double-diffusion plate (Fig. 3). A distinct precipitin line was obtained with antimouse IgM but not with anti-IgG or anti-IgA. The subclass specificity of YWK-II IgM was determined by the use of the peroxidase-linked immunosorbent assay with ABTS-H_20_2 as substrate (Table 1). Positive reactions were obtained with anti-μ (heavy chain) and anti-κ (light

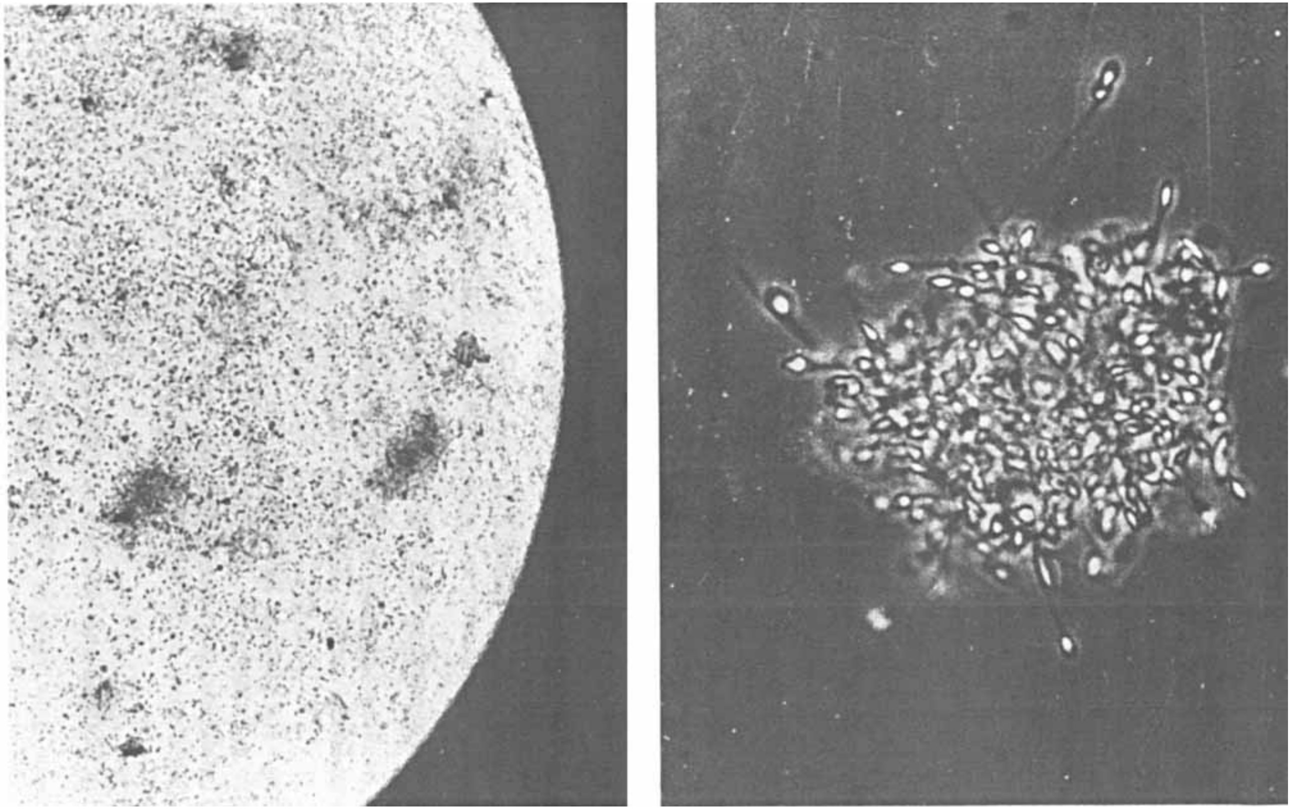

FIGURE 1 Sperm agglutination induced with YWK-II antibodies. Ascitic fluid diluted 1:30: Note mixed type of agglutination. 1A, ×40; 1B, ×800.

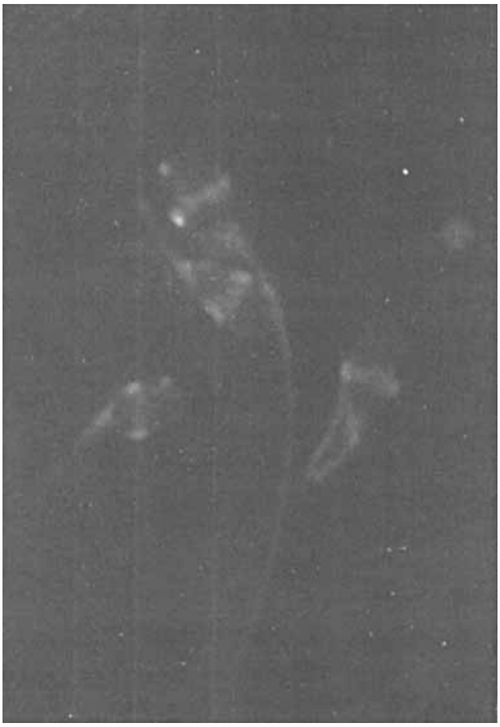

FIGURE 2 **Immunolocalization of YWK-II in human sperm. Sperm was incubated with YWK-II ascitic fluid (diluted 1:100) and subsequently with FITC-conjugated IgG fraction of goat antimouse Ig (diluted 1:100). Note staining over the equatorial region of the head, midpiece and tail. ×800.**

TABLE 1 **Determination of Subclass of YWK-II Monoclonal Antibody**[a]

Chain Type	Rabbit Antimouse Immunoglobulins (Subclass)	Subclass Specificity	Reaction
Heavy	IgA (Fc)	α	
Heavy	IgG_1	γ_1	—
Heavy	IgG_{2a}	γ_{2a}	—
Heavy	IgG_{2b}	γ_{2b}	—
Heavy	IgG_3	γ_3	—
Heavy	IgM (Fc)	μ	+
Light	kappa	κ	+
Light	lambda	λ	—

+ = positive reaction determined by ELISA, — = no reaction.

[a] Determined by enzyme-linked immunosorbent assay, as described in Materials and Methods.

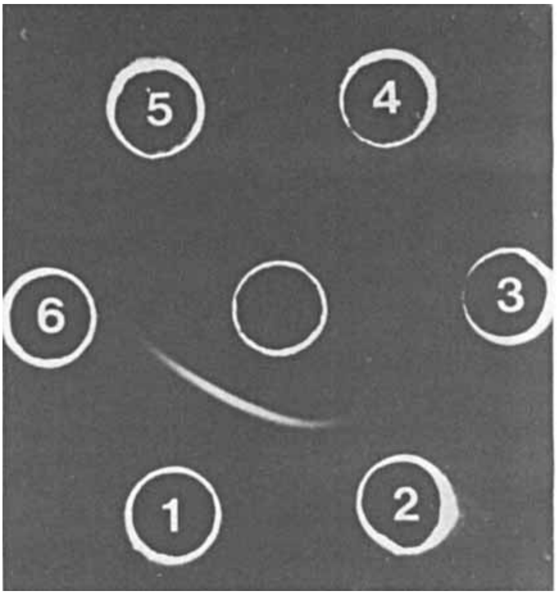

FIGURE 3 **Double diffusion analysis of purified MAB obtained from the culture medium of YWK-II cells on an Ouchterlony plate. The center well contains 20 μl of purified YWK-II immunoglobulin solution. The peripheral wells contain 20 μl each of the following antisera: (1) goat antimouse IgM; (2) goat antimouse IgM; diluted 1:10 (3) goat antimouse IgG; (4) goat antimouse IgG, diluted 1:10; (5) goat antimouse IgA; (6) goat antimouse IgA, diluted 1:10.**

chain). It can be concluded that the YWK-II immunoglobulin belongs to the IgM class and is comprised of μ heavy chains and κ light chains.

Purification and Identification of YWK-II Antigen

The sperm antigen interacting with YWK-II MAB was purified by immunoaffinity chromatography on Sepharose 4B-conjugated with YWK-II antibodies. The absorbed substance was eluted with 3.5 M KI. The purified sperm antigen was analyzed by SDS PAGE (Fig. 4). Two bands were separated: one major band with an estimated Mr 72 kD and a minor band of 60 kD.

Reactivity of YWK-II MAB with Sperm Protein

The reactivity of YWK-II with the sperm antigen purified by immunoaffinity chromatography was determined by the ELISA method (Fig. 5). The reaction was about 100-fold greater with the purified antigen compared with crude Triton X-100 extract of human sperm proteins.

Influence of YWK-II MAB on Sperm Penetration of Zona-Free Hamster Eggs

The penetration incidence of hamster eggs by sperm treated with YWK-II ascitic fluid was 52.2% compared with 95.1% for control sperm treated with SP 2/0 ascitic fluid (Table 2). The YWK-II MAB does not induce sperm agglutination under the conditions of the assay. Interference of sperm-egg interaction was observed. p Value was < 0.001 as determined by the X^2 test.

TABLE 2 Effect of YWK-II Monoclonal Antibody (MAB) on the Penetration of Hamster Egg by Human Sperm

MAB (Type)	Eggs Examined (No.)	Ova Penetrated (No.)	Penetration Incidence (%)
Control, SP 2/0 ascitic fluid	41	39	95.1
YWK-II ascitic fluid	23	12	52.2[a]

[a] p value < 0.001—compared with control by X^2 test.

DISCUSSION

MABs raised against human sperm proteins have been studied by several investigators [14, 17, 26, 33]. The YWK-II MAB and MA 5 MAB reported by Isahakia and Alexander [20] share common properties. Both MABs localized to the equatorial region of the sperm head and interacted with a sperm protein with an estimated Mr of 72 kD. The YWK-II MAB induced sperm agglutination and inhibited sperm penetration of zona-free hamster eggs, while the reported MA 5 MAB induced sperm immobilization. A MAB inducing sperm immobilization was reported by Shigeta et al. [35]. The estimated Mr of the interacting sperm antigen was 15 kD, suggesting that YWK-II, MA 5, and the sperm-immobilizing MAB are different entities.

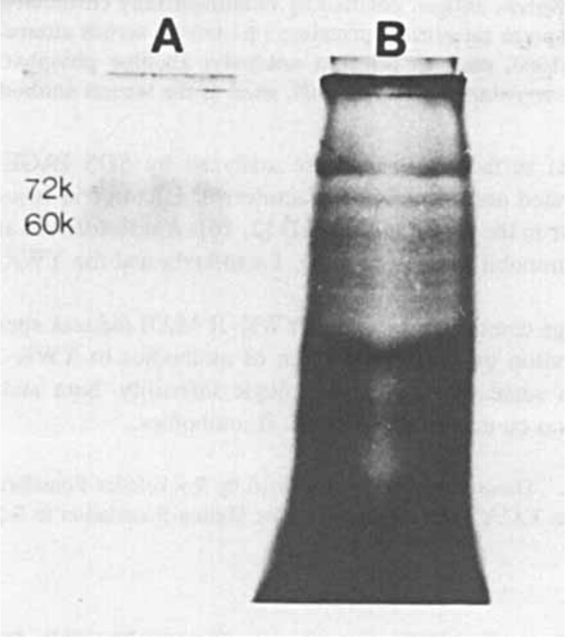

FIGURE 4 SDS PAGE pattern of human sperm membrane protein purified by immunoaffinity chromatography. Purification procedure is described in Materials and Methods. Proteins stained with silver reagent. Lane A, purified YWK-II sperm membrane; Lane B, crude Triton X-100 extract of human sperm proteins.

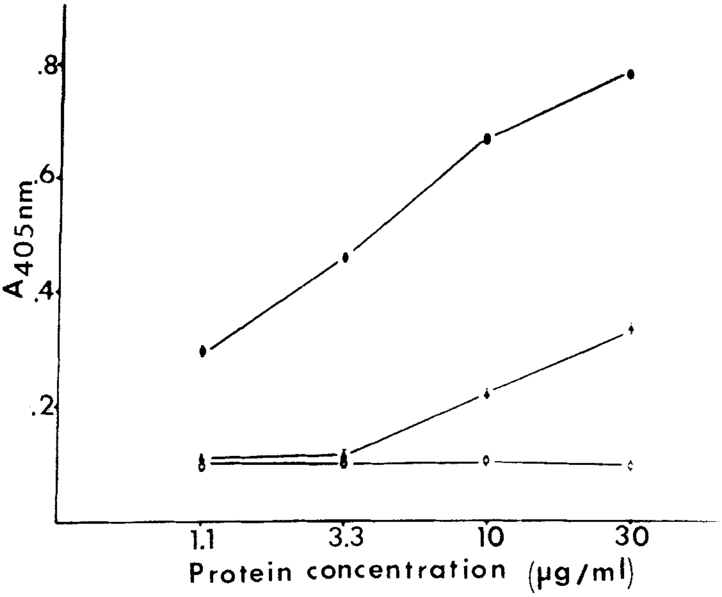

FIGURE 5 **Reactivity of YWK-II MAB with purified human sperm proteins determined by ELISA procedure. (•) Sperm antigen purified by immunoaffinity chromatography; (▲) Triton X-100 extract of human sperm membrane proteins; (○) bovine serum albumin (control). YWK-II ascitic fluid diluted 1:1000, used as the first antibody; alkaline phosphatase-conjugated sheep f(ab')$_2$ antimouse immunoglobulins diluted 1:500, used as the second antibody.**

Radiolabeled sperm surface proteins were analyzed by SDS PAGE [11]. A 72-76 kD component was separated and identified as lactoferrin. Lactoferrin is a spermatozoa-coating glycoprotein with a Mr in the range of 76-81kD [2, 16]. Antilactoferrin antibodies are capable of agglutinating and immobilizing sperm [16]. Lactoferrin and the YWK-II antigen might be related proteins.

The present findings demonstrate that the YWK-II MAB induces sperm agglutination and inhibits sperm penetration of eggs. Production of antibodies to YWK-II antigen may be a contributing factor in some cases of immunologic infertility. Sera and cervical fluid from infertile subjects should be analyzed for YWK-II antibodies.

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REFERENCES

1. Ackerman SB, Graff D, Van Uem JFHM, Swanson RJ, Veeck LL, Acosta AA, Garcia JE (1984): Immunologic infertility and in vitro fertilization. Fertil Steril 42:474-477
2. Aisen P, Listowsky I (1980): Iron transport and storage proteins. Annu Rev Biochem 49:357-393
3. Alexander NJ (1984): Antibodies to human spermatozoa impede sperm penetration of cervical mucus on hamster eggs. Fertil Steril 41:433-439
4. Beer AE, Neaves WB (1978): Antigenic status of semen from the viewpoints of the female and male. Fertil Steril 29:3-22

5. Biggers JD, Whitten WK, Whittingham DF (1971): The culture of mouse embryos *in vitro*. In: Methods of Mammalian Embryology. Daniel JC Jr (Ed.). San Francisco: W.H. Freeman, pp. 86-116
6. Bronson RA, Cooper GW, Rosenfeld DL (1982): Sperm-specific isoantibodies and autoantibodies inhibit the binding of human sperm to the human zona pellucida. Fertil Steril 38:724-729
7. Dor J, Rudak E, Aitken RJ (1981): Antisperm antibodies: Their effect on the process of fertilization studied in vitro. Fertil Steril 35:535-541
8. Etribi A, Ibrahim A, Mahmoud K, El-Haggar S, Hammada T, El-Ahmadi I. (1982): Antisperm antibodies and human infertility. Fertil Steril 37:236-239
9. Fjallbrant B (1968): Sperm agglutinins in sterile and fertile men. Acta Obstet Gynecol Scand 47:89-101
10. Franklin RR, Dukes CD (1964): antispermatozoa antibody and unexplained infertility. Acta Obstet Gynecol Scand 89:6-9
11. Goodman SA, Young LJ (1981): Immunological identification of lactoferrin as a shared antigen on radioiodinated human sperm surface and in radioiodinated human seminal plasma. J Reprod Immunol 3:99-108
12. Haas GG, Cines DB, Schreiber AD (1980): Immunological infertility: Identification of patients with antisperm antibody. N Engl J Med 303:722-727
13. Hass GG Jr, Sokoloski JE, Wolf DP (1980): The interfering effect of human IgG antisperm antibodies on human sperm penetration of zona-free hamster eggs. Am J Reprod Immunol 1:40-43
14. Hancock RJT, Faruki S (1985): Detection of antisperm activities of monoclonal antibodies to human sperm. J Reprod Immunol 7:215-223
15. Hargreave TB (1982): Incidence of serum agglutinating and immobilizing sperm antibodies in infertile couples. Int J Fertil 27:90-94
16. Hekman A, Rumke P (1968): The antigens of human seminal plasma (with special reference to lactoferrin as a spermatozoa-evading antigen). In: Protides of the Biological Fluids, Vol. 4, Peters H (Ed). New York: Pergamon pp 11-21
17. Hinrichsen-Kohane AC, Hinrichsen MJ, Schill WB (1985): Analysis of antigen expression on human spermatozoa by means of monoclonal antibodies. Fertil Steril 43:279-285
18. Husted S (1975): Sperm antibodies in men from infertile couples. Analysis of sperm agglutinins and immunofluorescent in 657 men. Int J Fertil 20:113-121
19. Ingerslev HJ, Hjort T (1979): Sperm agglutinating antibodies and β-sperm agglutinins in sera from infertile and fertile women. Fertil Steril 31:496-502
20. Isahakia M, Alexander NJ (1984): Interspecies cross-reactivity of monoclonal antibodies directed against human sperm antigen. Biol Reprod 30:1015-1026
21. Isojima S, Li TS, Ashitaka Y (1968): Immunologic analysis of sperm-immobilizing factor found in sera of women with unexplained sterility. Am J Obstet Gynecol 101:677-683
22. Isojima S, Koyama K, Tsuchiya K (1974): The effect on fertility in women of circulating antibodies against human spermatozoa. J Reprod Fertil (Suppl) 21:125-150
23. Jager S, Kremer J, Kuiken J, Van Slochteren-Draaisma T (1980): Immunoglobulin class of antispermatozoal antibodies from infertile men and inhibition of in vitro sperm penetration into cervical mucus. Int J Androl 3:1-14
24. Jones WR (1980): Immunologic infertility: Fact or fiction? Fertil Steril 33:577-586
25. Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685
26. Lee CG, Huang YS, Huang CH, Hu PC, Menge AC (1982): Monoclonal antibodies to human sperm antigen. J Reprod Immunol 4:173-181
27. Marmar JL, Praiss DE, De Benedicts TJ (1980): Modification of the Friberg microagglutination tray technique for detection of sperm antibodies. Arch Androl 4:347-352
28. Mathur S, Baker ER, Williamson HO, Derrick FC, Teague KJ, Fudenberg HH (1981): Clinical significance of sperm antibodies in infertility. Fertil Steril 36:486-494
29. McLaren A (1964): Immunological control of fertility in female mice. Nature (London) 201:582-585
30. Menge AC (1980): Clinical immunologic infertility: Diagnostic measure, incidence of antisperm antibodies, fertility and mechanisms. In: Immunological Aspects of Infertility and Fertility Regulation, Dhindsa DS, Schumacher GFB (Eds). New York: Elsevier North Holland, pp 205-224
31. Moghissi KS Sacco AG, Borin K (1980): Immunologic infertility I. Cervical mucus antibodies and postcoital test. Am J Obstet Gynec 136:941-950
32. Naz RK, Alexander NJ, Isahakia M, Hamilton MS (1984): Monoclonal antibody to a human germ cell membrane glycoprotein that inhibits fertilization. Science 225:342-344
33. Paul S, Baukloh V, Baillie M, Mettler L (1983): Generation of monoclonal antibodies against human spermatozoa and seminal plasma constituents. Clin Reprod Fertil 1:235-240
34. Rumke PH, Hellinga G (1959): Autoantibodies against spermatozoa in sterile men. Am J Clin Pathol 32:357-363

35. Shigeta M, Watanabe T, Maruyama S, Koyama K, Iosjima S (1980): Sperm-immobilizing monoclonal antibody to human seminal plasma antigens. Clin Exp Immunol 42:458–462
36. Towbin H, Staehelin T, Gordon J (1979):Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354
37. Tung KSK (1976): Antifertility vaccine: Considerations of their potential immunopathologic complications. Int J Fertil 121:197-206
38. Tyler A (1961): Approaches to the control of fertility based on immunological phenomenon. J Reprod Fertil 2:473–506
39. Yan YC, Wang LF, Mitsudo SM, Koide SS (1986): Characterization of an antisperm monoclonal antibody inducing human sperm agglutination. In: Immunological Approach to Contracepion and Promotion of Fertility, Talwar GP (Ed). New York: Plenum Press, pp 231-246
40. Yan YC, Wang LF, Koide SS (1986— Characterization of sperm agglutinating monoclonal antibody and purification of the human sperm antigen. Int J Fertil 31:77–85